GABA. In the presence of 10 μM progesterone (~ED_{20} concentration), the percentage of acrosome-reacted spermatozoa in response to 10 μM GABA increased, but not significantly, compared to GABA alone and this increase was much lower than a simple addition of effects, suggesting that progesterone and GABA are utilizing the same receptor and/or mechanism of action. To test this hypothesis, we evaluated the effects of progesterone on the acrosome reaction in the presence of increasing concentrations of bicuculline. Bicuculline suppressed, but not completely, progesterone-induced acrosome reaction, suggesting that this steroid interacts with the $GABA_A$ receptor to promote the acrosome reaction. Given that the $GABA_A$ receptor is linked to the chloride channel, we tested the effects of the chloride channel blocker picrotoxin on progesterone- or GABA-induced acrosome reaction. Picrotoxin suppressed completely the stimulatory effects of progesterone and only partially those of GABA on the acrosome reaction.

Conclusion: GABA stimulated the acrosome reaction through an activation of the $GABA_A$ and, to a lesser extent, the $GABA_B$ receptor. In addition, this study adds further evidence that progesterone acts also through a $GABA_A$-like, chloride channel-linked receptor to promote the acrosome reaction.

10.30–10.45

O-023. Influence of glucose on protein tyrosine phosphorylation in mouse spermatozoa

Urner F.[1] and Sakkas D.[2]

Clinic of Sterility, Department of Obstetrics and Gynecology, University Hospital of Geneva, 1211 Geneva 14, Switzerland and [2]Reproductive Biology and Genetic Group, Department of Medicine, University of Birmingham and Assisted Conception Unit, Birmingham Women's Hospital, Birmingham B15 2TG, UK

Introduction: The beneficial effect of glucose on gamete fusion in the mouse is probably mediated by its metabolism through the pentose phosphate pathway of the spermatozoa since spermatozoa supplemented with NADPH instead of glucose can fuse with the oolemma [Urner and Sakkas (in press) *Biol. Reprod.*]. As the phosphorylation state of proteins on tyrosine residues is enhanced by NADPH in human spermatozoa [Aitken *et al.* (1995) *J. Cell Sci.*, **108**, 2017–2025], and as protein tyrosine phosphorylation has been correlated with capacitation in mouse spermatozoa [Visconti *et al.* (1995) *Development*, **1212**, 1129–1137], we have examined the influence of glucose and NADPH on protein tyrosine phosphorylation in mouse spermatozoa.

Materials and methods: Mouse epididymal spermatozoa were released into 200 μl of M16 medium under oil and incubated as concentrated suspensions (40–80×10^6 spermatozoa/ml) or as diluted suspensions (5–10×10^6 spermatozoa/ml). Sperm proteins were solubilized and separated on sodium dodecyl sulphate–10% polyacrylamide gel, prior to being transferred to nitrocellulose membranes. Immunodetection of phosphotyrosines was performed using a monoclonal anti-phosphotyrosine antibody (AG10,UBI) and enhanced chemiluminescence detection technique.

Results: When diluted suspensions of spermatozoa were incubated in the presence of glucose, a set of proteins (mol. wt 45–100 kDa) became tyrosine phosphorylated after 90 min. In the absence of glucose, this increase was delayed and became visible only after 3 h of incubation. Following a 3 h incubation of spermatozoa as concentrated suspensions, tyrosine phosphorylation did not increase despite the presence of glucose, but, after subsequent removal of glucose and dilution in glucose-free medium, the addition of glucose (5.5 mM) or NADPH (5 mM) resulted in a rapid increase (5–15 min) in tyrosine phosphorylation. In addition, this increase correlated with the ability of spermatozoa to fuse with the oolemma.

Conclusion: Glucose is involved in the increase in protein tyrosine phosphorylation in mouse spermatozoa. The lag phase (90 min) between the release of epididymal spermatozoa into glucose-containing medium and the apparition of tyrosine phosphorylation suggests that a preparative phase is required to allow a glucose-dependent increase in tyrosine phosphorylation. The rapid induction of tyrosine phosphorylation by both glucose and NADPH in spermatozoa primed with glucose but prevented to undergo tyrosine phosphorylation is in favour of a specific effect of glucose/NADPH on the induction of tyrosine phosphorylation. Subsequently, the modulation of protein tyrosine phosphorylation by glucose, via its metabolism through the pentose phosphate pathway, appears to be crucial to the fertilization process by rendering the spermatozoa fusogenic.

10.45–11.00

O-024. Generation of monoclonal antibodies against a homologous synthetic ZP2 peptide

Hinsch K.-D., Gröger S., Schill W.-B. and Hinsch E.

Centre of Dermatology and Andrology, Justus-Liebig-University Giessen, Gaffkystr. 14, 35385 Giessen, Germany

Introduction: The zona pellucida (ZP) glycoprotein ZP2 is crucially involved in the process of fertilization. It has been suggested that ZP2 mediates secondary binding of spermatozoa via the inner acrosomal membrane. Human and mouse ZP2 proteins differ in the primary structure as derived from cDNA clones. Recently, we generated polyclonal antibodies against synthetic ZP2 peptides and demonstrated that one of the investigated ZP2 epitopes corresponds with a ZP2 domain that is important for secondary sperm–ZP binding. The aim of the study was to generate monoclonal antibodies (mAb) against this conserved synthetic ZP2 peptide and to identify ZP2 in mammalian ZP.

Materials and methods: Selection of the synthetic peptides was based on their probability of being antigenic sequences as predicted by analysis of the primary structure. Specificity of mAb and titres were determined by enzyme-linked immunosorbent assay. Using immunohistochemical methods, we sought to investigate if anti-ZP2 mAb react with human or bovine ZP2 protein.

Results: mAb ZP2-20 and mAb ZP2-24 specifically recognized the ZP2 peptide that was employed as immunogen. The